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ORIGINAL ARTICLE

The effects of Cinnamaldehyde on early brain injury and cerebral vasospasm following experimental subarachnoid hemorrhage in rabbits

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Abstract

The neuroprotective and vasodilatory effects of cinnamaldehyde have been widely studied and documented. On the basis of these findings, we hypothesized that cinnamaldehyde exhibits therapeutic effects on subarachnoid hemorrhage-induced early brain injury and cerebral vasospasm. Thirty-two adult male New Zealand white rabbits were randomly divided into four groups of eight rabbits: control, subarachnoid hemorrhage, subarachnoid hemorrhage + vehicle, and subarachnoid hemorrhage + cinnamaldehyde. An intraperitoneal dose of 50 mg/kg cinnamaldehyde was administered 5 min following an intracisternal blood injection, followed by three further daily injections at identical doses. The animals were sacrificed 72 h after subarachnoid hemorrhage was induced. The cross-sectional areas and arterial wall thicknesses of the basilar artery were measured and hippocampal degeneration scores were evaluated. Treatment with cinnamaldehyde was effective in providing neuroprotection and attenuating cerebral vasospasm after subarachnoid hemorrhage in rabbits. It effectively increased the cross-sectional areas of the basilar artery and reduced the arterial wall thickness; in addition, hippocampal degeneration scores were lower in the cinnamaldehyde group. The findings of this study showed, for the first time to our knowledge, that cinnamaldehyde exhibits neuroprotective activity against subarachnoid hemorrhage-induced early brain injury and that it can prevent vasospasm. Potential mechanisms underlying the neuroprotection and vasodilation were discussed. Cinnamaldehyde could play a role in subarachnoid hemorrhage treatment.

Keywords Cinnamaldehyde · Early brain injury · Neuroprotection · Rabbit · Subarachnoid hemorrhage · Vasospasm

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Introduction

Subarachnoid hemorrhage (SAH) is a catastrophic disease that results in a range of neurological injuries from minor cognitive impairments to major disabling cerebral infarctions and even death (Miller et al. 2014). The two chief causes of poor prognosis after initial bleeding are early brain injury (EBI) and cerebral vasospasm (Song et al. 2013a). The initial bleeding can result in EBI during the first 72 h following SAH (Hasegawa et al. 2011). There is substantial evidence of neuroinflammation following SAH, which can cause cerebral edema and cell death (Miller et al. 2014). Following SAH, there is an imbalance between the intrinsic antioxidant systems and the production of reactive oxygen species (ROS), and oxidative stress, which can trigger apoptosis, also plays an important role in the development of EBI (Ayer et al. 2008). The exact mechanisms underlying cerebral vasospasm remain unclear.

Proposed causative mechanisms include neuroinflammation, oxidative stress, imbalance between vasoconstrictors and vasodilators, neuronal mechanisms that regulate vascular tone, apoptosis, and endothelial proliferation (Kolias et al. 2009).

Cinnamaldehyde (CA), a phenylpropanoid, is a primary ingredient of cinnamon. It reportedly exhibits various therapeutic properties (Xue et al. 2011), including antitumoral, antipyretic, antimicrobial, antidiabetic, antimutagenic, antioxidant, and anti-inflammatory activities (Chew et al. 2010; Kim et al. 2006; Koh et al. 1998; Lv et al. 2017; Nour et al. 2018; Sharma et al. 2018; Shaughnessy et al. 2001; Xue et al. 2011). Numerous studies have shown that CA exhibits potent neuroprotective activity, inhibiting neuroinflammation, oxidative stress, and apoptosis (Bae et al. 2018; Chen et al. 2016; Fu et al. 2017; Ho et al. 2013; Lv et al. 2017; Pyo et al. 2013; Shi et al. 2017; Yang et al. 2016; Zhao et al. 2015). Its vasodilator effects have also been widely studied and demonstrated in various vessels, such as the mouse ear artery (Aubdool et al. 2016), rat aorta (Alvarez-Collazo et al. 2014; Xue eet al 2011; Yanaga et al. 2006), mesenteric artery (Veras et al. 2013), and porcine coronary artery (Raffai et al. 2014), as well as in cerebral arteries (Earley et al. 2009).

On the basis of these neuroprotective and vasodilatory activities, we hypothesized that CA may have therapeutic effects on SAH-induced EBI and cerebral vasospasm.

Materials and methods

Experimental groups

Adult male New Zealand white rabbits ($n = 32$) weighing 3100–3650 g were randomly divided into four groups ($n = 8$ each): the control, SAH, vehicle, and CA groups. The control group underwent sham surgery wherein SAH was not induced. After the induction of anesthesia, the cisterna magna was punctured as described, 1 ml/kg of cerebrospinal fluid (CSF) was removed, and 1 ml/kg of saline (0.9% NaCl) was slowly injected. Cerebral vasospasm was induced in all the animals in the other three groups, as described in the following paragraph. The SAH group received no treatment. The vehicle group received an intraperitoneal dose of 1 ml/kg 0.5% Tween 80 saline solution (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) once every 24 h for 72 h. The first dose was administered 5 min following the intracisternal blood injection. The CA group was administered an intraperitoneal dose of 50 mg/kg CA (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) dissolved in 1 ml/kg 0.5% Tween 80 saline solution once every 24 h for 72 h. The first dose was administered 5 min following the intracisternal blood injection. The dosage of CA was selected on the basis of previously published articles (Huang et al. 2007; Zhao et al. 2015).

Anesthesia and the surgical procedure

The animals were kept at an appropriate room temperature (18 °C–21 °C) and fed a standard diet ad libitum with a 12 h light–dark cycle. Prior to the surgical procedure, the animals were anesthetized with the intramuscular administration of a combination of 70 mg/kg ketamine (Ketalar, Pfizer, Turkey) and 5 mg/kg xylazine (Rompun, Bayer, Turkey). Spontaneous breathing continued throughout the procedure. Arterial blood samples for measuring PO_2 and PCO_2 were obtained from catheterized auricular arteries; only animals with $PO_2 >$ 70 mmHg and $PCO_2 < 40$ mmHg were included in this study. The auricular arterial catheters were also used for measuring the heart rate and arterial blood pressure during the procedure. The core body temperature was rectally monitored and maintained at 37 °C ± 0.5 °C using a heater.

The cerebral vasospasm model

The model used in this study is one of the most commonly used protocols for establishing SAH in rabbits (Gürer et al. 2014; Kertmen et al. 2012, 2014). With the rabbit in the prone position, its head was flexed and the atlanto-occipital membrane was exposed via a midline nuchal incision and dissection of the paravertebral muscles. A 25-gauge needle was inserted through the dura mater and the arachnoid membrane into the cisterna magna; 1 ml/kg of CSF was withdrawn and within 2 min the same volume of fresh, non-heparinized autologous arterial blood obtained from the auricular arterial catheter was injected into the cisterna magna. The animal was then placed in 30° head-down position for 30 min to hold the blood in the basal cistern. After the rabbit recovered from anesthesia, its vital signs were confirmed and it was returned to its cage for the establishment of EBI and cerebral vasospasm.

Perfusion fixation

The animals were sacrificed 72 h after the procedure perfusion fixation. After anesthetizing the animals as described, the auricular artery was catheterized for blood pressure monitoring and blood gas analysis. After satisfactory respiratory parameters were obtained, a thoracotomy was performed, the left ventricle was cannulated, the right atrium was opened widely, and the descending aorta was clamped. After perfusion with 300 ml of physiological saline (0.9% NaCl), 200 ml of 10% formaldehyde was perfused as a fixative. The perfusion was performed at a standard height of 100 cm from the chest. The brain was then removed and stored in formaldehyde solution overnight at 4 °C.

Histological morphometric analysis of the basilar artery

Vasospasm assessment in rabbits was performed by measurement of basilar artery lumen area as previously described (Vellimana et al. 2017, Zhou et al. 2007a, 2007b). The brainstem was embedded in paraffin and the entire basilar artery was sectioned into five segments, each 2 mm in length (Fig. 1), which were stained with hematoxylin and eosin (H&E). Morphometric measurements were taken on all five segments using a Bs200ProP Image Processing and Analysis System (BAB, Ankara, Turkey).

The luminal area was measured as the area bounded by the internal elastic lamina. The measurements for the five segments were averaged to obtain the cross-sectional area (CSA) for each basilar artery. The mean ± SD value for each basilar artery was used as the final CSA value for that particular vessel.

The arterial wall thickness (AWT) for the wall between the lumen and the external border of the muscular layer was measured for four quadrants of each segment of the basilar artery. If the luminal border was undulating, an extra measurement was taken from the internal elastic lamina to the external border of the muscular layer. The AWT for each basilar artery was obtained by averaging these measurements. The mean ± SD value obtained from each basilar artery was used as the final AWT value for that particular vessel.

All the measurements were made two times for each artery in a blinded fashion by two pathologists and the final values were obtained by averaging these measurements.

Hippocampal degeneration

The hippocampus of each rabbit was embedded in paraffin, sectioned into 4–6 μm thick slices and stained with H&E. Morphological signs of neuronal degeneration, such as neuronal shrinkage, hyperchromasia, and nuclear pyknosis were evaluated under a light microscope. The presence and extent of hippocampal degeneration in the CA1, CA3, and dentate gyrus regions were semiquantitatively scored as follows: 1, normal appearance; 2, a few degenerated neurons among normal neurons; 3, a large number of degenerated neurons with some scattered normal neurons; 4, complete degeneration with no residual normal neurons (Gürer et al. 2014; Kertmen et al. 2012, 2014). The sum of the scores for the three regions of the hippocampus was considered to be the degeneration score, and the mean values were used in statistical analysis.

Statistical analysis

Data analysis was performed using GraphPad Prism 6 for Windows (GraphPad Software, La Jolla, CA, USA). Whether continuous variables were normally distributed was determined by using the Shapiro–Wilk test. Levene's test was used for evaluating the homogeneity of variances. Data are

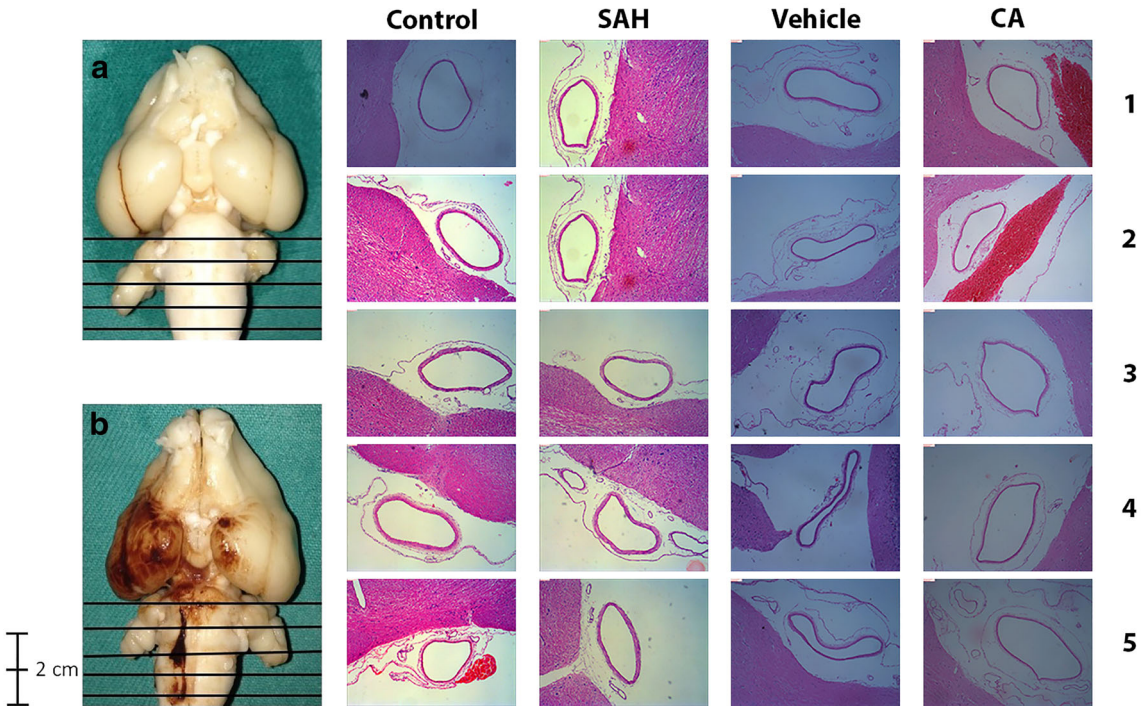

Fig. 1 Macroscopic views of the bases of rabbit brains obtained from the control group (**a**) and the SAH group (**b**). Representative histological cross-sections of the basilar artery corresponding with all study groups (H&E stain; magnification 40×). SAH: subarachnoid hemorrhage; CA: cinnamaldehyde

presented as the mean ± SD, median (IQR), or median (range), as appropriate. The mean differences among groups were compared using one-way ANOVA, and the Kruskal–Wallis test was used for comparisons of median values. A p value less than 0.05 was considered statistically significant.

Results

Morphometric analysis of the basilar artery

In the control group, the mean CSA for the basilar artery was 308,045.9 ± 28,394.6 μm^2. In the SAH and vehicle groups, it has decreased to 109,601.9 ± 17,874.7 μm^2 and 103,715.8 ± 25,808.5 μm^2, respectively (both $p < 0.001$ in comparison with the control group). No statistically significant difference was noted in mean CSA between the SAH and vehicle groups ($p = 0.984$). In the CA group, the mean basilar artery CSA was 227,098.6 ± 46,542.8 μm^2, which was significantly increased compared with the SAH and vehicle groups ($p < 0.001$ and $p < 0.01$, respectively) (Fig. 2a).

The mean AWT of the basilar artery was 15.84 ± 1.96 μm in the control group, 42.86 ± 2.94 μm and 43.44 ± 8.55 μm in the SAH and vehicle groups, respectively, and 24.9 ± 4.4 μm in the CA group. Compared with the control group, the mean AWT was significantly higher in the SAH and vehicle groups (both $p < 0.001$). Treatment with CA reduced the mean AWT significantly when compared with both the SAH and the vehicle groups (both $p < 0.001$). No significant difference in AWT was noted between the SAH and vehicle groups ($p = 0.996$) (Fig. 2b). The mean basilar artery CSA and AWT values are presented in Table 1.

Table 1 The mean CSA and AWT of the basilar artery regarding for groups

Groups	CSA	AWT
Control	308,045.9 (28,394.6)[b,c]	15.84 (1.96)[b,c]
SAH	109,601.9 (17,874.7)[b,d]	42.86 (2.94)[b,d]
Vehicle	103,715.8 (25,808.5)[c,e]	43.44 (8.55)[c,f]
CA	227,098.6 (46,542.8)[d,e]	24.9 (4.4)[d,e]
p value [a]	F = 64.37, <0.001	F = 55.98, <0.001

CSA cross-sectional area, *AWT* arterial wall thickness, *SAH* subarachnoid hemorrhage, *CA* cinnamaldehyde

[a] One-Way ANOVA

[b] Control vs SAH ($p < 0.001$)

[c] Control vs Vehicle ($p < 0.001$)

[d] SAH vs CA ($p < 0.001$)

[e] Vehicle vs CA ($p < 0.01$)

Pathological examination of the hippocampus

Light microscopic examination of the CA1, CA3, and dentate gyrus samples of the hippocampus were normal for the control group (Fig. 3), but almost complete degeneration of the neurons was observed in the SAH and vehicle groups (Figs. 4 and 5, respectively). The pathological appearance was clearer for the CA group, with fewer degenerated neurons with hyperchromasia, neuronal shrinkage, and nuclear pyknosis (Fig. 6).

The mean degeneration score was 3.8 ± 0.8 for the control group, 11.3 ± 0.7 and 11.0 ± 0.7 for the SAH and vehicle groups, respectively, and 7.5 ± 0.7 for the CA group. The scores for the SAH and vehicle groups were significantly higher than that for the control group (both $p < 0.001$). The degeneration score for the CA group was significantly lower than those for the SAH and vehicle groups (both $p < 0.001$) (Fig. 2c). The pathological examination values are summarized in Table 2.

Discussion

EBI and cerebral vasospasm are the most important clinical contributors to SAH-induced disability and death (Barrow et al. 2018; Han et al. 2017a, b). EBI occurs within the first

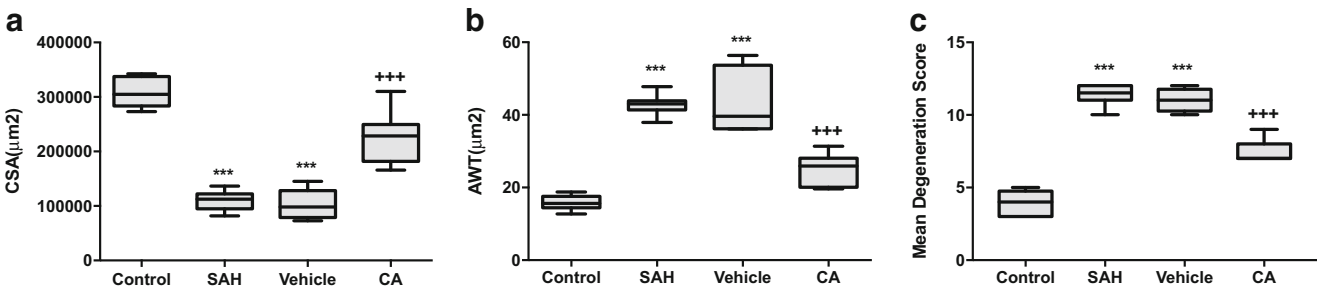

Fig. 2 Mean basilar artery cross-sectional areas (**a**) and wall thicknesses (**b**) of the study groups, and the hippocampal degeneration scores (**c**). The horizontal lines in the middle of each box indicate the median values; the top and bottom borders of the boxes indicate the 25th and 75th percentiles, respectively. The whiskers above and below the box indicate the maximum and minimum levels. *** $p < 0.001$ compared to the control group. +++ $p < 0.001$ compared to the vehicle group. CA: cinnamaldehyde; CSA: cross-sectional area; Deg.: degeneration; SAH: subarachnoid hemorrhage

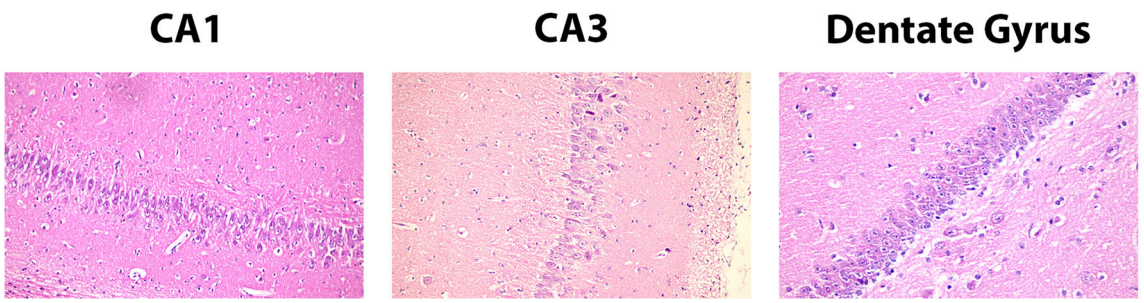

Fig. 3 Photomicrographs of slices from the CA1, CA3, and dentate gyrus of the hippocampus from the control group, showing a normal appearance. (H&E stain; magnification 20–40×)

72 h of the initial aneurysm rupture and is a strong determinant of the patient's prognosis (Cahill et al. 2006; Sehba et al. 2011). Studies have demonstrated that inflammation, oxidative stress, and apoptosis are associated with EBI after SAH (Barrow et al. 2018; Cahill et al. 2006; Han et al. 2017a, 2017b; Sehba et al. 2011). Furthermore, the breakdown products of blood in the cisternal spaces can cause delayed and sustained vasospasm of the large diameter arteries, resulting in catastrophic consequences due to ischemic neurologic deficits (Boulouis et al. 2017; Diringer and Zazulia 2017). The underlying pathological mechanism of SAH-induced vasospasm is multifactorial, complex, and not fully understood. Various recent studies have focused on strategies for preventing or treating both SAH-induced EBI and vasospasm (Gürer et al. 2014; Kertmen et al. 2012, 2014; Miller et al. 2014).

Cinnamon oil is obtained from the stem bark of *Cinnamomum cassia* and has been widely used in food as a flavoring agent and as a traditional medicine in Asia (Wijesekera 1978). CA which is the major constituent of cinnamon oil exhibits a wide range of biological activities, including antipyretic, antitumoral, antibacterial, antidiabetic, anti-inflammatory, and antioxidant activities (Chew et al. 2010; Kim et al. 2006; Koh et al. 1998; Lv et al. 2017; Nour et al. 2018; Sharma et al. 2018; Shaughnessy et al. 2001; Xue et al. 2011; Zhu et al. 2017). It also reportedly exerts neuroprotective effects against cerebral ischemia (Chen et al. 2016; Zhao et al. 2015), glutamate excitotoxicity (Lv et al. 2017), and neurodegenerative diseases such as Alzheimer's and Parkinson's diseases (Bae et al. 2018; George et al. 2013), and it also has anti-neuroinflammatory effects on neuronal cell cultures (Bae et al. 2018; Fu et al. 2017; Ho et al. 2013; Shi et al. 2017; Yang et al. 2016), as well as vasorelaxant effects on the isolated rat aorta (Alvarez-Collazo et al. 2014; Xue et al. 2011; Yanaga et al. 2006), mesenteric artery (Veras et al. 2013), mouse ear artery (Aubdool et al. 2016), and porcine coronary artery (Raffai et al. 2014), and in cerebral arteries (Earley et al. 2009). Because of these neuroprotective and vasodilatory effects, we hypothesized that CA potentially exerts protective effects against SAH-induced EBI and vasospasm.

Inflammation remains one of the most significant mechanisms of EBI after SAH (Miller et al. 2014). Elevated levels of inflammatory cytokines such as tumor necrosis factor-α and interleukin-1 and interleukin-8 have been observed following SAH (Fassbender et al. 2001; Kim et al. 2006). Animal studies have shown that blocking inflammatory pathways protect neurons from SAH-induced cell death (Caner et al. 2012; Yatsushige et al. 2007). In clinical studies, C-reactive protein, which is a reliable marker of inflammation, has been shown to increase following SAH and to be associated with EBI and a poor outcome (Alvarez-Collazo et al. 2014; Zhong et al. 2017). These findings leave no doubt that inflammation plays an important role in EBI. Furthermore, elevated levels of inflammatory cytokines in the CSF, serum, and cerebral arterial wall have been shown to correlate with the severity of vasospasm (Fassbender et al. 2001; Gaetani et al. 1998b; Hendryk et al. 2004; Muroi et al. 2013). Various reports have concluded that CA inhibits neuroinflammation in numerous animal models (Chen et al. 2016; Fu et al. 2017; Ho et al. 2013; Pyo et al. 2013; Zhao et al. 2015).

There is substantial evidence suggesting that oxidative stress plays a major triggering role in the development of EBI after

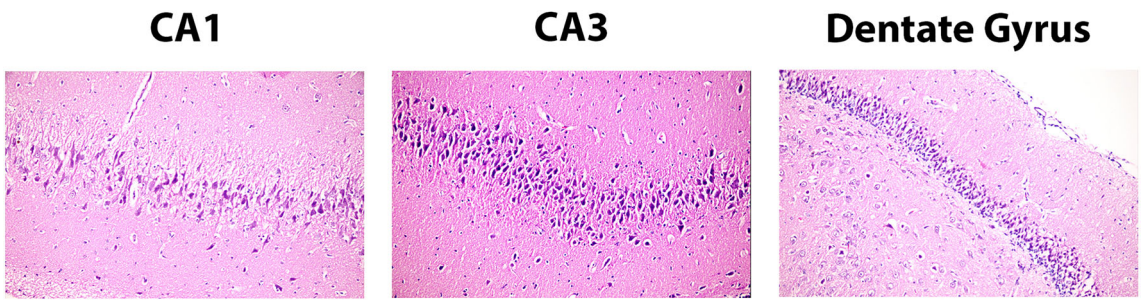

Fig. 4 Photomicrographs of slices from the CA1, CA3, and dentate gyrus of the hippocampus from the SAH group showing complete degeneration of the neurons. (H&E stain; magnification 20–40×). SAH: subarachnoid hemorrhage

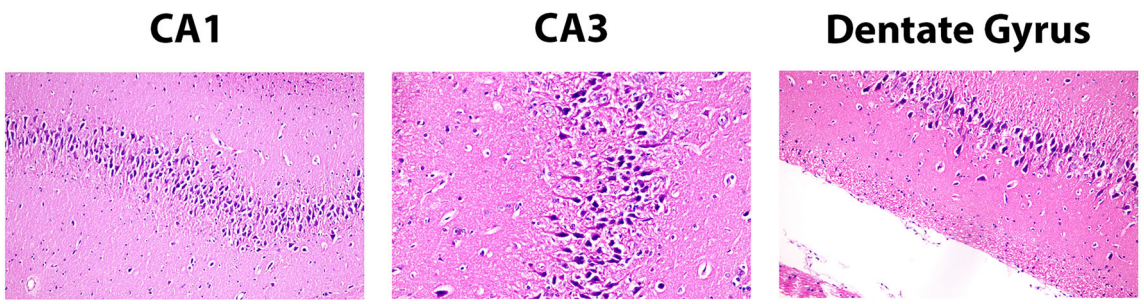

Fig. 5 Photomicrographs of slices from the CA1, CA3, and dentate gyrus of the hippocampus from the vehicle group (1 ml/kg 0.5% Tween 80 saline solution) showing complete degeneration of the neurons. (H&E stain; magnification 20–40×)

SAH (Han et al. 2017a, b), and that it could be a novel target for the potential treatment of SAH-induced neuronal injury (Sehba and Bederson 2006). Following SAH, the increased oxidative stress inhibits the intrinsic antioxidant enzyme systems (such as superoxide dismutase and glutathione peroxidase), resulting in an imbalance between the production of ROS and the antioxidant effects of those systems (Ayer and Zhang 2008; Endo et al. 2007; Han et al. 2017a, 2017b). This imbalance results in neuronal damage (Gaetani et al. 1998a; Zhuang et al. 2012). CA is a well-known antioxidant agent (Foti et al. 2004; Lv et al. 2017; Yang et al. 2016), and animal studies have shown that its administration increased superoxide dismutase activity and reduced malondialdehyde and nitric oxide levels (Davaatseren et al. 2017; Sehba and Bederson 2006).

Neurons are very sensitive to oxidative stress and apoptosis, which are both associated with the development of neurological deficits (Ayer and Zhang 2008; Zhang et al. 2015). The oxidative stress induced by SAH irreversibly triggers apoptotic pathways in a large number of neuronal cells, and it has been confirmed that SAH-induced apoptosis plays a role in the mechanisms underlying the development of EBI (Ayer and Zhang 2008; Han et al. 2017b; Zhang et al. 2015). Lv et al. (2017), reported evidence of the antiapoptotic activity of CA against the glutamate-induced apoptosis of rat adrenal pheochromocytoma cells.

The findings of the present study showed that CA exerted a neuroprotective effect against SAH-induced hippocampal nerve degeneration, an indicator of EBI, in a SAH rabbit model. These protective effects may be the result of the anti-neuroinflammatory, antioxidant, and antiapoptotic activity of CA demonstrated in previous studies.

As a secondary messenger, intracellular calcium (Ca^{+2}) plays critical roles in a wide range of physiological processes, including smooth muscle contraction (Clapham 1995). Arterial smooth muscle contraction depends on Ca^{+2}, and its influx through Ca^{+2} channels is one of the most important regulators of vascular smooth muscle contraction (Nelson et al. 1990); however, this influx can cause vasospasm (Hai and Murphy 1988; Knot and Nelson 1998). Following SAH, the membrane potential depolarization and increased activity of L-type voltage-dependent Ca^{+2} channels (VDCC) result in an increase in intracellular Ca^{+2} concentration, leading to vasospasm (Koide et al. 2011; Song et al. 2013b). The role of VDCC activity in SAH-induced vasospasm is apparent in clinical practice, with L-type Ca^{+2} channel blockers such as nimodipine, and verapamil used in the prophylaxis and treatment of vasospasm (Kamp et al. 2012; Narayan et al. 2018; Stuart et al. 2011). Alvarez-Callazo et al. (2014), concluded that CA exerted its inhibitory effect through L-type Ca^{+2} channels and that this effect contributed to its vasorelaxant action. The inhibition was dose-dependent and occurred in a pulsed manner, similar to the action of the classic Ca^{+2} channel blocker verapamil (Alvarez-Callazo et al. 2014; Rubio et al. 1993). In addition, CA has been shown to induce the endothelium-independent relaxation of vascular smooth muscle in isolated porcine coronary arteries by inhibiting Ca^{+2} sensitivity and influx (Raffai et al. 2014).

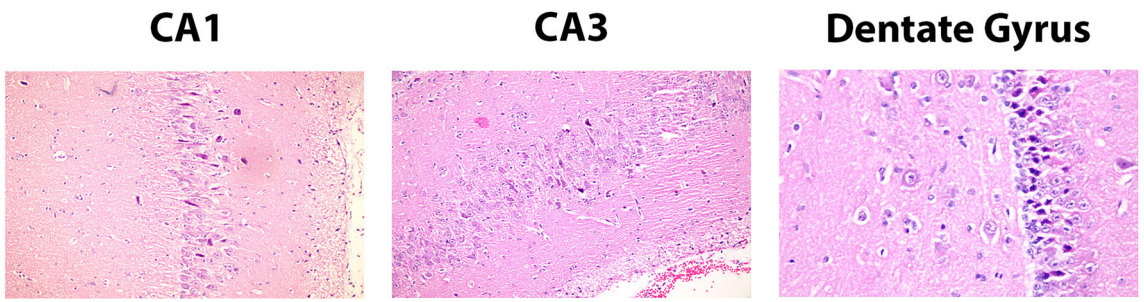

Fig. 6 Photomicrographs of slices from the CA1, CA3, and dentate gyrus of the hippocampus from the CA group (50 mg/kg CA), showing only mild degeneration of the neurons. (H&E stain; magnification 20–40×). CA: cinnamaldehyde

Table 2 Hippocampal degeneration scores regarding for groups

Variables	Control	SAH	Vehicle	CA	p value [a]
Dentate Gyrus	1 (1–2)[b,c,d]	4 (3–4)[b,e]	4 (3–4)[c,f]	3 (1–4)[d,e,f]	F = 27.89, <0.001
CA1	1 (1–2)[b,c,d]	4 (3–4)[b,g]	4 (3–4)[c,h]	2 (2–3)[d,g,h]	F = 61.09, <0.001
CA3	1 (1–2)[b,c,d]	4 (3–4)[b,g]	4 (2–4)[c,i]	2.5 (2–3)[d,g,i]	F = 32.15, <0.001
Mean Deg. Score	4 (3–5)[b,c,d]	11.5 (10–12)[b,g]	11 (10–12)[c,h]	7 (7–9)[d,g,h]	F = 164.4, <0.001

SAH subarachnoid hemorrhage, *CA* cinnamaldehyde, *Deg.* degeneration

[a] One-Way ANOVA

[b] Control vs SAH ($p < 0.001$)

[c] Control vs Vehicle ($p < 0.001$)

[d] Control vs Cinnamaldehyde ($p < 0.001$)

[e] SAH vs Cinnamaldehyde ($p < 0.01$)

[f] Vehicle vs Cinnamaldehyde ($p < 0.05$)

[g] SAH vs Cinnamaldehyde ($p < 0.001$)

[h] Vehicle vs Cinnamaldehyde ($p < 0.001$)

[i] = Vehicle vs Cinnamaldehyde ($p < 0.01$)

After the discovery of transient receptor potential ankyrin-1 (TRPA1), studies have focused on its role in regulating peripheral vascular tone (Aubdool et al. 2016; Earley et al. 2009; Pozsgai et al. 2010; Sullivan et al. 2015). TRPA1 is found on the endothelium of cerebral arteries (Sullivan et al. 2015), and its activation in rat cerebral arteries results in vasorelaxation (Earley et al. 2009). It has been demonstrated that CA is one of the most potent agonists of the TRPA1 channels (Bodkin and Brain 2011; Raffai et al. 2014; Vriens et al. 2008), and that its administration causes TRPA1-dependent vasodilatation (Aubdool et al. 2016; Pozsgai et al. 2010). Aubdool et al. (2016), reported that CA can activate TRPA1, and the release of calcitonin gene-related peptide, a dilatator of cerebral arteries (Edvinsson et al. 1987; Flynn et al. 2017), further mediates vasodilatation.

Tromboxane A_2 (TXA_2) is a well-documented, potent vasoconstrictor and an important modulator of vascular tone (Cockerham et al. 1991). Elevated levels of TXA_2 in the CSF following SAH have been reported (Pickard et al. 1994). SAH induces the upregulation of TXA_2 receptors in cerebrovascular muscle cells, and this has been shown to correlate with the reduction of cerebral blood flow (Ansar et al. 2010). Takenaga et al. (Sullivan et al. 2015), reported that CA reduced the formation of TXA_2.

Both neuroinflammation and the formation of ROS reportedly play important roles in the development of SAH-induced vasospasm (Kolias et al. 2009). As previously described, CA has anti-inflammatory (Chen et al. 2016; Fu et al. 2017; Ho et al. 2013; Pyo et al. 2013; Zhao et al. 2015) and antioxidant (Alvarez-Collazo et al. 2014; Davaatseren et al. 2017; Foti et al. 2004) activities. In the present study, we investigated the effects of CA on SAH-induced vasospasm. This revealed that treatment with CA had a protective effect on SAH-induced vasospasm in rabbits. As described in this study, the cerebral vasodilatory action of CA occurs via multiple mechanisms, including VDCC blockage, TRPA1 agonism, release of calcitonin gene-related peptide, TXA_2 inhibition, anti-neuroinflammation, and antioxidation.

The results of this study suggested that CA has beneficial effects on protecting the brain from EBI and SAH-induced vasospasm. However, the study had some limitations. First of all, the current study is a first step in this line of studies, to investigate the possible effects of CA in SAH-induced vasospasm and EBI. The preferred drug administration time was 5 min after the establishment of SAH. The aim of this early administration time of the drug was to reach the effective blood concentration as soon as possible, before the establishment of vasospasm. For more clinically relevant results, further studies with different drug administration times are needed. The value of the study could be enhanced by dose-dependent results with a delayed histopathological assessment of the vasospasm after SAH. In addition, biochemical parameters, immunohistochemical and molecular biology analysis are required for a better understanding of the potential underlying mechanisms of CA associated with its neuroprotective and vasodilatory actions. The study also lacked functional outcome measures with neurological examinations. Numerous patients are admitted to hospital several days after initial bleeding with already-established vasospasm; further investigations are required to ascertain whether CA can reverse already-established vasospasm. In addition, further research is required for investigating the effect of CA for both the prevention and reversal of vasospasm.

Conclusion

This study showed, for the first time to our knowledge, that CA can prevent vasospasm via neuroprotective activity

against SAH-induced EBI. The possible underlying mechanisms of neuroprotection and vasodilatation were discussed. CA could play a role in the treatment of SAH. However, further studies based on our findings may be helpful in evaluating this promising medication for SAH-induced EBI and vasospasm.

Compliance with ethical standards

Conflict of interest All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Ethical approval The animal care and all the experimental procedures complied with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes during experimental use. All the experimental procedures in this study were reviewed and approved by the local committee for animal ethics.

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